# The impact of seasonal and year-round transmission regimes on the evolution of influenza A virus

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**Summary**

Punctuated antigenic change is believed to be a key element in the evolution of influenza A; clusters of antigenically similar strains predominate world-wide for several years until an antigenically distant mutant emerges and instigates a selective sweep. It is thought that a region of East-Southeast Asia with year-round transmission acts as a source of antigenic diversity for influenza A and seasonal epidemics in temperate regions make little contribution to antigenic evolution. We use a mathematical model to examine how different transmission regimes affect the evolutionary dynamics of influenza over the lifespan of an antigenic cluster. Our model indicates that, in non-seasonal regions, mutants that cause significant outbreaks appear before the peak of the wildtype epidemic. A relatively large proportion of these mutants spread globally. In seasonal regions, mutants that cause significant local outbreaks appear each year before the seasonal peak of the wildtype epidemic, but only a small proportion spread globally. The potential for global spread is strongly influenced by the intensity of non-seasonal circulation and coupling between non-seasonal and seasonal regions. Results are similar if mutations are neutral, or confer a weak to moderate antigenic advantage. However, there is a threshold antigenic advantage, depending on the non-seasonal transmission intensity, beyond which mutants can escape herd immunity in the non-seasonal region and there is a global explosion in diversity. We conclude that non-seasonal transmission regions are fundamental to the generation and maintenance of influenza diversity due to their epidemiology. More extensive sampling of viral diversity in such regions could facilitate earlier identification of antigenically novel strains and extend the critical window for vaccine development.

## Introduction

Influenza A viruses are responsible for regular epidemics, and occasional pandemics, throughout the world (1). From 1968 until the recent emergence of a new pandemic influenza A (H1N1) virus the majority of infections have been caused by the H3N2 subtype (1-3). Rapid but low fidelity replication facilitates genetic diversification in the viral population. Mutations affecting the virus surface proteins may result in less efficient recognition by protective antibodies and necessitate regular updates of the influenza vaccine (4-6). Genetic changes accumulate continuously in the influenza genome. Antigenic change, however, appears to take the form of punctuated jumps interspersed with small scale drift (7-9); clusters of antigenically similar strains persist for several years until an antigenically distant strain emerges to found a replacement cluster. Modeling studies suggest that the punctuated aspect of the evolutionary pattern can be explained by broad short-term cross-immunity (10-11) or antigenic landscapes composed of 'neutral' networks (12). In the latter case, following the founding of a new antigenic cluster, antigenically neutral or nearly neutral drift mutations accumulate, exploring the sequence space until an antigenically distant mutant emerges to seed a new cluster that replaces the existing one.

In temperate regions influenza incidence is seasonal. There is a pronounced epidemic peak in winter and infections are rarely observed in summer (13-14). Influenza incidence is less variable in tropical and subtropical regions and, over the course of a whole year, the total number of infections is believed to be similar in all regions (15). Phylogenetic analysis has suggested that temperate epidemics are probably reseeded every year from an external source (2, 16-17) and revealed little evidence for positive selection over the course of temperate region influenza seasons (17). Early phylogenetic studies suggested that many new antigenic variants of influenza emerge in China and neighbouring countries (18-19). A more recent study suggested that seasonal influenza epidemics are started each year by viruses imported from a region of East-Southeast Asia, and implied that the majority of antigenic evolution occurs in this region (20). This area includes countries with tropical and subtropical climates; local oscillations in incidence are relatively small and poorly synchronized, resulting in year-round transmission on a regional scale.

Here we use a mathematical model to examine how seasonal and non-seasonal transmission regimes, and their global interplay, influence the evolution of influenza over the three to five year periods between large punctuated antigenic jumps, which we do not model. A mutant with a

large antigenic advantage relative to circulating strains will experience a relatively large susceptible population. As such, the system is not at equilibrium and epidemic infection dynamics are expected even if the underlying transmission rate is seasonally invariant. Within this transient epidemiological context we focus on the impact of antigenically neutral and nearly neutral mutations but, where pertinent, also explore the implications of more significant antigenic changes. We start with simple intuitive models, and gradually build up layers of insight to arrive at a fuller understanding of the various mechanisms at work, and how they fit together in more complex models.

**Mathematical model**

Our model is based on a conventional extension of the standard SIR framework to multiple co-circulating strains with immune cross-reaction (7, 10, 12, 21-33). The global population is divided into three distinct regions (Fig. 1), each of population size $N$. Two regions are characterized by transmission and non-transmission seasons that each last six months and do not overlap. The underlying probability of transmission intensity $\beta$ is zero in the non-transmission season and positive in the transmission season, changing sinusoidally with a maximum of $(1 + \delta)\beta$ at the midpoint of the season. In the third region transmission is constant year-round. In each region individuals make transmissible contact with other individuals in the same region at rate $\beta$, and with individuals in other regions at rate $\tau_{ZW}\beta$ where $\tau_{ZW}$ is much less than 1. The seasonal transmission regions are directly coupled to each other by $\tau_{SS}$, and coupled with the non-seasonal transmission region by $\tau_{YS}$. Infected individuals recover at rate $\gamma$. Natural mortality occurs at rate $\mu$, and an equal birthrate ensures the population size remains constant.

The interaction of multiple strains is modeled using a history-based approach. Hosts are classified according to the virus strains with which they are currently infected, and those with which they have been previously infected. Virus strains are defined by a fifty element binary genotype. Forty elements are phenotypically neutral, ten determine antigenic similarity. Immunity reduces susceptibility. Hosts who have recovered from an infection acquire permanent complete immunity to the infecting strain and partial immunity to other strains. There is no temporary immunity. The antigenic Hamming distance between two strains $h$ is the number of antigenic bitstring locations at which they are different. Cross-immunity $g$ is linearly related to the Hamming distance $g(h) = \min\{\sigma h, 1\}$ where $0 < \sigma < 1$ is the antigenic advantage associated with a single point mutation. Nearly neutral mutations are represented by values of $\sigma$ close to 0. If a host has experienced more

than one previous infection, the immune response is determined by the previous strain most closely related to the challenging strain.

The start point of our time-frame is the emergence of a mutant, termed the wildtype, that is antigenically distant from previously circulating strains. We approximate this circumstance with the assumption that the entire population is susceptible. Consequently, the epidemiological dynamics are transient in all regions. In the non-seasonal transmission region there is a single wildtype epidemic lasting several years (Fig S1). In the seasonal transmission regions, there are sequences of epidemics over several years. The epidemics end due to stochastic fade out when the susceptible population is sufficiently depleted. Generally, the time until fade-out is longer if the transmission intensities are lower. The model is iterated as a discrete population, continuous time Markov process using the Gillespie algorithm (34). Full details can be found in the Supplementary Information.

## Results

*Decoupled regions, wildtype and up to one mutant*

Here we consider individual, decoupled, regions to assess the effects of seasonal and non-seasonal transmission on epidemic potential. Classically, the epidemic potential of a pathogen is expressed by the basic reproductive number $R_0$, the number of secondary infections resulting from a single infected individual in an otherwise naive population. If transmission is non-seasonal, $R_0 = \beta N/(\gamma+\mu)$. (35-36). If transmission is seasonal, the expected number of secondary infections depends on the time at which the infected individual is introduced, and is termed the effective reproductive number $R_e(t) = \beta(t)N/(\gamma+\mu)$. For basic models, branching process arguments give expressions for the probability that a single infected individual causes a significant outbreak (37-39). In order to compute corresponding probabilities using our model we define an 'epidemic' to be at least 50 simultaneous infections. When transmission is non-seasonal, the time of the initial infection does not affect the probability of an epidemic. When transmission is seasonal, even though $R_e(t) > 1$ and each infection is expected to lead to more than one secondary infection throughout the transmission season, infections introduced in the latter half of the season rarely lead to a significant outbreaks (Fig. S2). Immediately after introduction the number of infections increases slowly. A declining transmission rate exacerbates this effect and the end of the season curtails transmission before the epidemic gains momentum.

As the wildtype strain circulates immunity accumulates in the host population. The epidemic potential of a second, mutant, strain depends on the size of the immune classes in the population and their susceptibility to re-infection. Mutant epidemics, defined as at least 50 simultaneous infections, will usually manifest as outbreaks within the wildtype epidemic. As the wildtype epidemic progresses, the probability that a single neutral or nearly neutral mutant strain will cause an epidemic decreases (Fig. 2). If transmission is non-seasonal, this decrease becomes rapid as the wildtype epidemic approaches its peak. If transmission is seasonal the decrease is less pronounced because wildtype epidemics are curtailed by the end of each season, before the susceptible population is exhausted, and immunity accumulates more slowly. Furthermore, the mid-season peak in the transmission rate may allow an infected individual to infect several others, even when there is extensive immunity in the population. Mutants in seasonal regions are most likely to be successful if they are introduced around the second month of the transmission period. The success rate of earlier mutants is reduced by the low transmission rate. Later mutants are compromised by the low transmission rate, accumulating host immunity and the brevity of the remaining transmission season. Relatively large antigenic advantages – for our parameter set re-infection probabilities of up to 0.6 - have only a weak impact on the probability that a mutant will be successful. However, when the re-infection probability exceeds around 0.6, mutant epidemic probabilities increase rapidly and become almost independent of the wildtype dynamics (Fig. S2).

*Coupled regions, wildtype and one mutant*

Here we assess the effects of coupling on mutant epidemics, persistence and global spread using the model with two seasonal transmission regions and one non-seasonal transmission region. We consider a wildtype, introduced at time $t = 0$, and a single mutant strain with a probability $\sigma$ of re-infecting individuals immune to the wildtype. Coupling affects the wildtype epidemic behavior in all regions. Stronger direct coupling between the seasonal regions increases the magnitude of the seasonal epidemics (Fig. S3). Stronger coupling between the seasonal and non-seasonal regions increases the magnitude of epidemics in all regions (Fig. S4). The mutant strain is introduced into this epidemiological context at a random time. We define a mutant epidemic in a region to be at least 50 simultaneous infections.

A nearly neutral mutant introduced to the seasonal region is more likely to cause an epidemic in that region than in other regions (Fig. 3a). If it does escape from the seasonal region, it usually becomes

established in all regions, which we term cosmopolitan. Mutants are only likely to spread to other regions if they are introduced in the first year of the wildtype epidemic (Figs. 3b-d), near the start of the transmission season (Fig. S5). In the second year, the probability that a mutant becomes established locally is 20-40% lower than the first year, and the probability that it spreads elsewhere is 80-90% lower. Stronger coupling between seasonal and non-seasonal transmission regions increases the probability that the seasonal region mutant becomes cosmopolitan. A nearly neutral mutant introduced to the non-seasonal region has a lower probability of becoming established locally than a mutant in the seasonal region. However, a large proportion of locally successful mutants become cosmopolitan (Fig. 3e). Mutants are most likely to spread to other regions if they are introduced some time during the first year of the wildtype epidemic (Figs. 3f-h, S5). In the second year, the probability that a mutant becomes established locally is 60% lower, and the probability that it spreads elsewhere is 95% lower. Stronger coupling between the seasonal and non-seasonal transmission regions has little impact unless close to zero, in which case it increases the probability that non-seasonal mutants become cosmopolitan.

Regardless of the region into which the mutant is introduced the direct coupling between seasonal regions has little impact (Fig. S6). Results are similar if the mutant is neutral, has a small or intermediate antigenic advantage (Figs. S5 – S9). However, the overall probability that a non-seasonal mutant becomes cosmopolitan begins to increase rapidly when the re-infection probability exceeds approximately 0.5 (Fig. S10). This increase is driven by mutants introduced in the second and third years having a much higher chance of becoming established.

*Coupled regions, multiple spontaneous mutants*

Here we extend the coupled model to allow mutant strains to emerge spontaneously. Initially the wildtype strain is introduced and an epidemic commences. Each element of the dominant viral genotype associated with each infected individual has a small probability of switching as long as that individual is infected. Each difference in the antigenically relevant section of the genotypes confers a re-infection probability of $\sigma$, up to a maximum of 1.

When the antigenic advantage of each mutation is nil ($\sigma = 0$), weak ($\sigma = 0.1$) or intermediate ($\sigma = 0.3$), mutants are more likely to be successful, i.e. cause significant outbreaks, in the region where they originate than elsewhere (Figs. 4a,b, S11). In the seasonal region, the majority of all mutants appear in the first year of the wildtype

epidemic, when the number of wildtype infections is highest. Most of these mutants appear in the second half of the transmission season. Nevertheless, a large proportion of all mutants that cause significant outbreaks appear in the first half of the transmission season (Fig. S16, S17). In the non-seasonal region, the majority of all mutants appear almost two years into the wildtype epidemic, again when the number of wildtype infections is highest, but the majority of successful mutants appear six months before this.

In general, mutants arising in the non-seasonal region are more likely to spread globally than mutants arising in seasonal regions. Stronger coupling between non-seasonal and seasonal regions increases the probability that mutants will spread globally, weakly for seasonal mutants, more strongly for non-seasonal mutants (Fig. 4a,b). If coupling is stronger, the majority of successful non-seasonal mutants still appear before the majority of all mutants, although everything happens earlier.

Stronger coupling causes the majority of all non-seasonal mutants to appear earlier, but still after the majority of successful mutants. A larger proportion of seasonal mutants appear in the second year, but again after a large proportion of the successful mutants in that year (Fig. S17). Stronger coupling between seasonal regions has little impact (Figs. S12, S17). The amplitude of the transmission rate fluctuations determines, to some extent, whether or not seasonal epidemics occur at all. Consequently, low amplitude fluctuations can severely limit the epidemic potential or wildtype and mutant strains alike. Away from this region, however, the amplitude of seasonal fluctuations has little clear impact on the epidemic potential of mutant strains (Figs. S13, S17).

If mutation is neutral or weakly advantageous, higher non-seasonal transmission intensity leads to weak increases in the probabilities that seasonal or non-seasonal mutants will be successful in the non-seasonal region (Figs. S14, S18). If mutation has an intermediate advantage, the probability of a significant outbreak increases rapidly when non-seasonal transmission intensity exceeds a certain threshold, in this case $R_0 = 1.3$, and there is an explosion in diversity. Similarly, if $R_0$ is fixed, greater antigenic advantages conferred by each mutation have little impact until the re-infection probability reaches a certain threshold, in this case around 0.5 (Fig. 4c.d). Then the probability that non-seasonal mutants cause significant outbreaks locally, and in seasonal regions, begins to increase rapidly until, at $\sigma = 0.7$, there is an explosion in diversity. Weak to intermediate antigenic advantages

have little impact on the phase lag between the appearance of the majority of successful mutants and the majority of all mutants (Fig. S18). A large antigenic advantage, however, leads the majority of successful non-seasonal mutants to appear in phase with the majority of all mutants.

Viral diversity, expressed transiently in terms of the number of extant genotypes, and cumulatively in terms of the total number of genotypes appearing over several years, is generally similar if the antigenic advantage of mutation is nil, weak or intermediate. Transient diversity is proportional to the number of infected individuals (Fig. S19). Cumulative diversity depends mainly on transmission intensity, again an indicator for the number of infected individuals, and most mutants arise directly from the wildtype (Fig. S20). However, when the re-infection probability associated with each mutation exceeds a certain threshold, which depends on the intensity of non-seasonal transmission, diversity begins to increase rapidly in the non-seasonal region. If the re-infection probability increases further, there is an explosion in diversity.

**Discussion**

We have used a mathematical model to investigate how seasonal and non-seasonal transmission paradigms influence the evolution of influenza A between the relatively large, punctuated, antigenic jumps. A study based on a deterministic model of a single epidemic with non-seasonal transmission found that longer epidemics facilitate greater antigenic drift, and that the majority of this drift occurs before the epidemic peaks (24). In the context of a spatially structured host population with multiple coupled transmission paradigms and viral evolution in a high dimensional antigenic space, we have shown that the epidemiology of regions with non-seasonal transmission makes them central to viral evolution, while the role of seasonal regions is limited by their epidemiology. In particular:

(i) In non-seasonal regions, mutant strains with the highest chance of causing significant local outbreaks emerge before the wildtype epidemic peaks – earlier than the majority of mutants. A large proportion of these mutants become cosmopolitan. After the epidemic peak, the effective reproductive number of mutants is low, even if they have a moderate antigenic advantage, and they do not benefit from the epidemic momentum enjoyed by the wildtype.

(ii) In seasonal regions, the mutant strains with the highest chance of causing significant local outbreaks, throughout the global wildtype epidemic, emerge in the first half of the transmission season - earlier than the majority of mutants. However, these mutants are unlikely to

spread globally unless they emerge at the start of the first season of the wildtype epidemic. The seasonal fluctuation in transmission intensity introduces a strong founder effect and allows mutants to temporarily escape the effects of herd immunity. However, mutants must appear early for an outbreak to build momentum before the transmission season ends.

(iii) The extent of migration between non-seasonal and seasonal transmission regions strongly influences whether mutant strains become cosmopolitan, wherever they first arise. Throughout the transmission periods there is continuous potential migration between non-seasonal and seasonal regions. Strains arising in seasonal regions can persist by migrating to non-seasonal regions. Strains circulating year-round have multiple opportunities to migrate to seasonal regions at the beginning of a transmission period when it is easier to found an epidemic.

(iv) Direct migration between out of phase seasonal transmission regions has little impact on the global spread of mutant strains. The overlap is brief, the epidemic in the donor region is winding down and colonization of the destination region is difficult because the transmission rate is low at the beginning of the season. The accumulation of host immunity accentuates this effect as it effectively shortens the transmission seasons, reducing the overlap.

(v) The probability that mutants cause significant outbreaks is similar if all mutations are antigenically neutral, or some mutations confer a weak to moderate antigenic advantage. However, after the antigenic advantage conferred by mutation exceeds a certain threshold, there is a rapid increase in the probability of significant outbreaks in the non-seasonal region and an explosion in the global viral diversity. This threshold depends on the intensity of non-seasonal transmission. In the non-seasonal region, most mutants appear around the epidemic peak, but by this time there is extensive immunity in the population. Therefore, the epidemic potential of the majority of mutants only increases significantly when their antigenic advantage allows them to escape the herd immunity at, and beyond, this point of the wildtype epidemic.

Our insights are based on numerical solutions of stochastic, discrete population, continuous time models. In order to maintain computational efficiency we used relatively small population sizes and a coarse spatial structure with clearly defined seasonal and non-seasonal transmission paradigms. We expect larger, more structured populations to lead to non-seasonal epidemics that build more gradually and last longer as immunity accumulates more slowly. This change of intensity may make it easier for multiple strains to co-

circulate. Allowing low-level transmission throughout seasonal 'non-transmission' periods may enhance the persistence of mutants in these regions. Additional regions with less clearly defined transmission paradigms may lead to increased migration between the well-defined seasonal and non-seasonal transmission regions. Weak localised seasonality in subregions of 'non-seasonal' regions may introduce some disruptive noise into the pattern of circulation and evolution. Assessing the impact of these factors is an important area for future research. The results presented here provide a baseline for comparison. Given that our results are consistent across a range of model complexities we do not, however, expect them to be substantially modified by any of these additional factors.

Our analysis indicates that one key characteristic of a source region is consistency of transmission. Factors often considered to enhance the regularity and duration of transmission include large host populations and a regional population structure composed of well-connected but semi-autonomous patches (40-42). A second key characteristic of a source region is strong connectivity to seasonal regions. A pronounced founder effect means that a strain introduced at the start of the seasonal transmission period is likely to dominate for the whole winter. Even antigenically advantaged mutants must be introduced in the first half the of the transmission period if they are to cause a significant outbreak. Regions with year-round transmission and strong global connectivity are much more likely to supply these founders. Genetic surveillance has suggested that most seasonal epidemics are started by viral seeds from East-Southeast Asia (2, 18-20). Asynchronous sub-regional epidemic patterns, high population density and widespread exchange with temperate region populations suggest that epidemiological conditions in this region are ideal for a source of antigenic novelty. As more epidemiological data become available from the tropics and subtropics it should be possible to identify further potential source regions.

Our analysis also indicates that antigenically novel mutants that rise to global predominance are likely to appear in a region with a stable transmission pattern, from lineages established some time before the peak of the epidemic associated with the currently predominant antigenic cluster. Extensive sampling of viral genetic diversity in regions identified as likely evolutionary sources will be required to identify such strains. Real-time phylogenetic monitoring, in combination with analysis of antigenic and epidemiological data, may then aid the earlier detection of novel antigenic variants. Improving our understanding of the epidemiology of influenza throughout tropical

and subtropical regions, and integrating this information with evolutionary analysis, is thus essential to improve predictions of the antigenic cluster transitions that are critical for vaccine efficacy.

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**Figures**

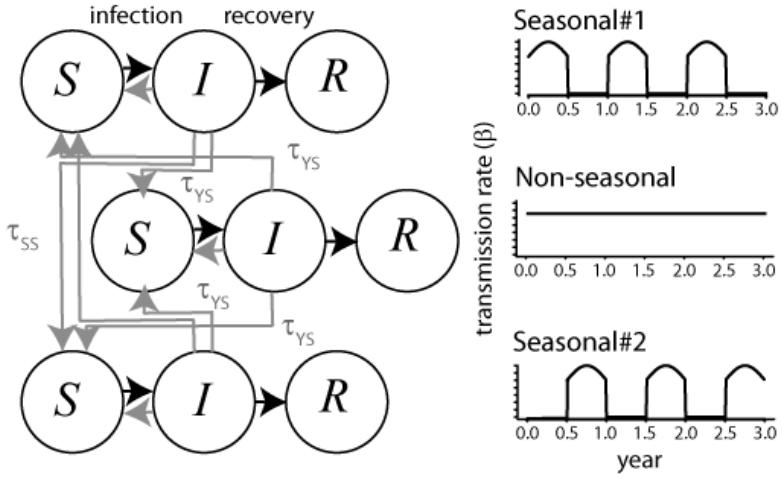


**Fig. 1:** Schematic model diagram. The global population is divided into three regions. Transmission is seasonal in two regions, year-round in the third. Each population consists of susceptible ($S$), infected ($I$) and recovered ($R$) individuals. Black lines indicate the flow between these states. Grey lines indicate transmission interaction.

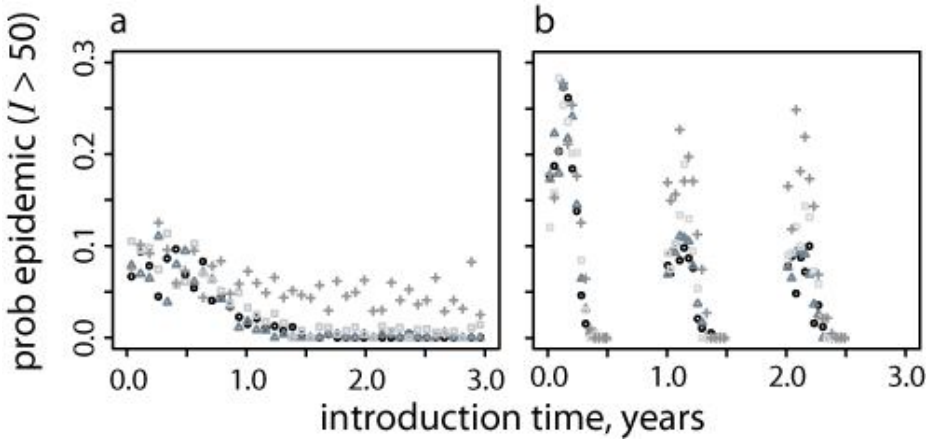


**Fig. 2:** Probabilities, as a function of introduction time, that a significant outbreak (at least 50 simultaneous infections) results from the introduction of one individual infected with a mutant strain into a population experiencing a wildtype epidemic. a – non-seasonal transmission, b - seasonal transmission. Circles, black: mutant strain has no antigenic advantage ($\sigma = 0$). Triangles, mid-grey: mutant can re-infect individuals immune to wildtype with probability $\sigma = 0.1$. Squares, pale grey: $\sigma = 0.3$. Pluses: $\sigma = 0.7$. Each set of points is based on 10000 independent trials with random introduction times. Initially all hosts susceptible except for 10 individuals with wildtype infections. Parameters values as in Table S1.

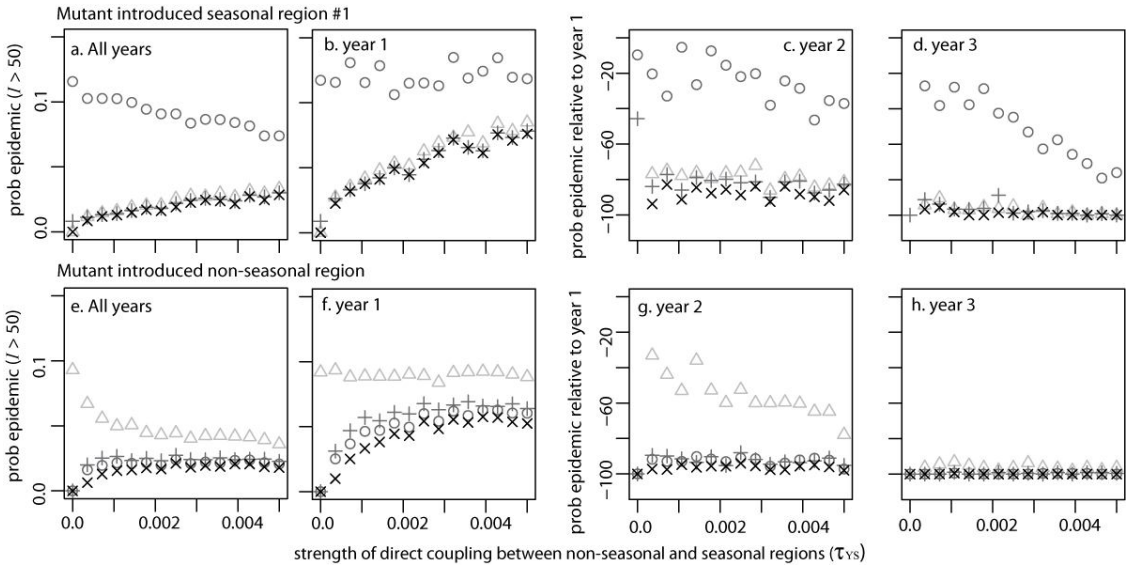


**Fig. 3:** Probabilities, as a function of coupling between seasonal and non-seasonal regions, that a significant outbreak results from the introduction of one individual infected with a mutant strain into a population experiencing a wildtype epidemic. Top row: mutant introduced in seasonal region #1. Bottom row: mutant introduced in non-seasonal region. **a, e**. Total probability over all introduction times in the first four years of the wildtype epidemic. **b, f.** Probability if the mutant strain is introduced in year 1. **c, d, g, h**. Probability if the mutant is introduced in year 2 or 3, expressed as the percentage change relative to year 1. Circles: probability of mutant epidemic in seasonal region #1. Triangles: non-seasonal region. Pluses: seasonal region #2. Crosses: all regions. Each point is the result of 10000 independent trials. Initially all hosts susceptible except for 10 individuals with wildtype infections in seasonal region #1. Parameter values as in Table S1, except $\sigma = 0.1$. No mutation.

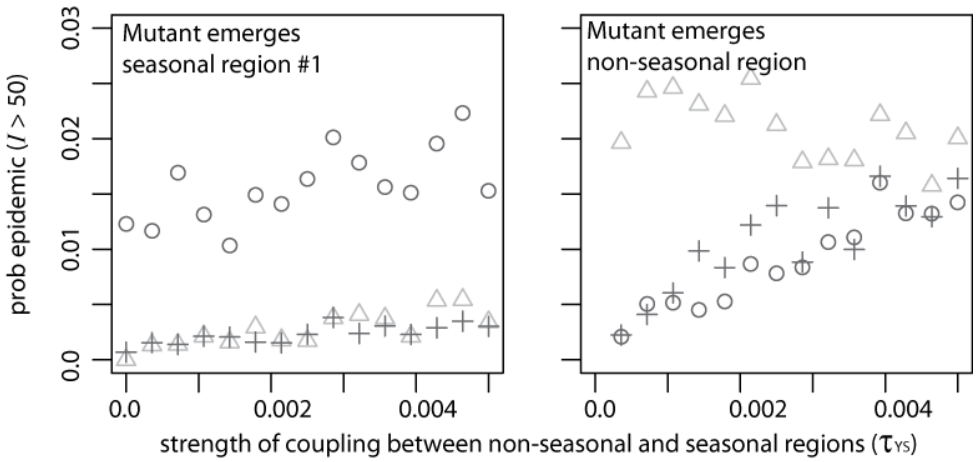

**Fig. 4:** Probabilities that mutant strains emerging spontaneously from existing strains cause epidemics. **a, b**: Depending on the strength of coupling between seasonal and non-seasonal regions when each difference between antigenic genotype sections translates into a re-infection probability of $\sigma$ = 0.1. **c, d**: Depending on the re-infection probability ($\sigma$) conferred by each difference between antigenic genotype sections when the coupling between seasonal and non-seasonal regions is intermediate $\tau_{YS}$ = 0.0005. **a, c**. Mutant strains emerging in seasonal region #1. **b, d**. Mutant strains emerging in non-seasonal region. All panels show the probability that the mutant causes at least 50 simultaneous infections in: circles - seasonal region #1, triangles – non-seasonal region, pluses – seasonal region #2. Each point is the result of 100 independent trials. Initially all hosts susceptible except for 10 individuals with wildtype infections in seasonal region #1. Parameter values as in Table S1.

**Supplementary Information**
**The impact of seasonal and year-round transmission regimes on the evolution of influenza A virus**

Adams and McHardy

## Supplementary Figures



## Detailed Model Description

**References**
**Figures**

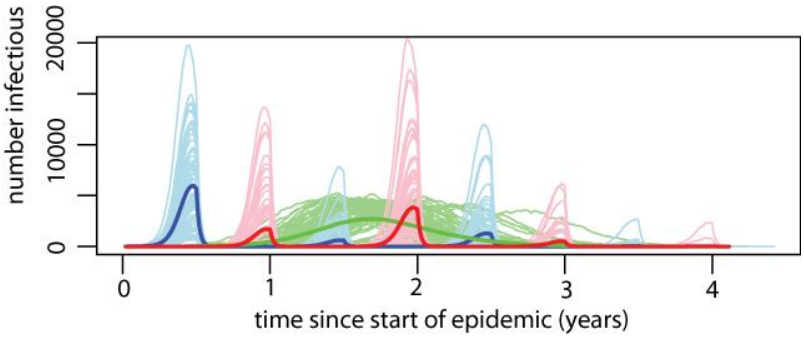


Figure S1: Wildtype epidemics. Number of infected individuals over a four year period following the introduction of the wildtype strain into a naive population. There is no mutation. Pale lines represent 100 independent realisations of the model. Dark lines are the means of these realisations after removing any trial for which the epidemic did not persist for at least 6 months. Blue: number of infectious individuals in seasonal region #1. Green: non-seasonal region. Red: seasonal region #2. Initially all populations were naive except 10 infected individuals in seasonal region #1. Parameter values as in Table S1. Seasonal epidemics peak toward the end of the transmission period, show high variability in magnitude and little overlap between regions. Non-seasonal epidemics build and decline slowly, show variability in timing but not magnitude, and peak after an average of just under two years.

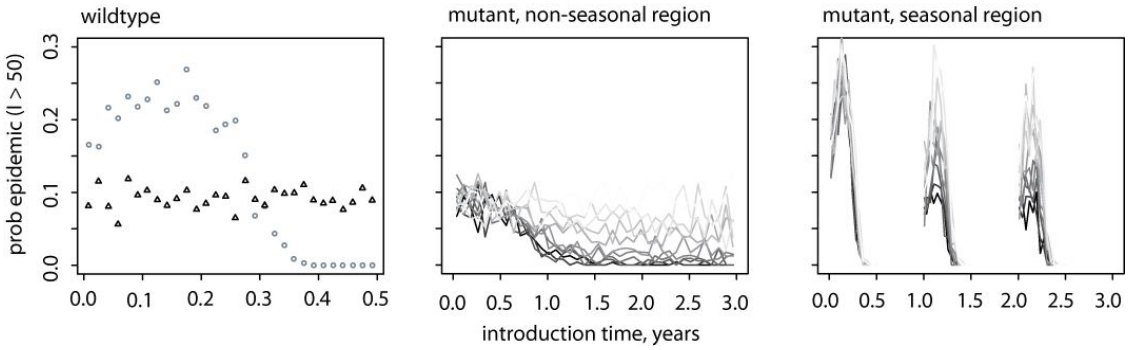


Figure S2: Probabilities, as a function of introduction time, that at least 50 simultaneous infections result from the introduction of a single infected individual. Left panel: Individual infected with wildtype strain introduced into a naive population. Triangles: non-seasonal transmission. Circles: seasonal transmission. Centre and right panels: Individual infected with mutant strain introduced into a population experiencing a wildtype epidemic resulting from 10 infected individuals at time 0. Centre panel: non-seasonal region. Right panel: seasonal region. The antigenic advantage of the mutant ranges from the capacity to re-infect none of the individuals immune to the wildtype ($\sigma = 0$, black line) to the capacity to re-infect all immune individuals ($\sigma = 1.0$, palest grey line). Each set of points is the result of 10000 independent trials with random introduction times. Parameters values as in Table S1. In the non-seasonal region the epidemic probability shows a weak response to antigenic advantages of up to 60% re-infection but increases significantly above this threshold. The pattern is similar in the seasonal region, but less clearly defined because immunity accumulates more slowly.

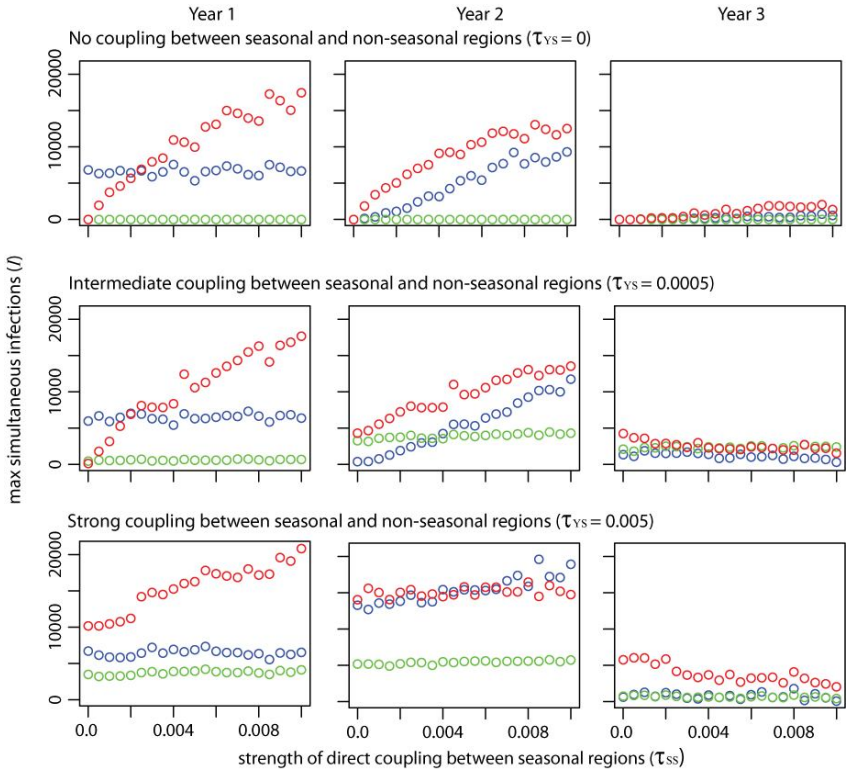


Figure S3**:** Epidemiological impact of direct coupling between seasonal regions. Maximum number of simultaneous wildtype infections in each of the three years following introduction, as a function of the strength of direct coupling between seasonal regions. Top row – no direct coupling between seasonal and non-seasonal regions. Middle row – intermediate coupling between seasonal and non-seasonal regions. Bottom row – strong coupling between seasonal and non-seasonal regions. Blue: maximum number of simultaneous infections in seasonal region #1. Green: non-seasonal region. Red: seasonal region #2. The model is composed of all three regions and a single virus strain. Each point is the mean of 100 independent realizations after removing any trial for which the epidemic did not persist for at least 6 months. Initially all populations were naive except 10 infected individuals in seasonal region #1. Parameter values as in Table S1. Stronger direct coupling between seasonal regions increases the mean size of epidemics in seasonal regions in the first two years unless coupling with the non-seasonal region is strong. Direct coupling between seasonal regions has little impact on the epidemiological dynamics in the non-seasonal region. Epidemic size may decrease in the third year because immunity has accumulated more rapidly. Seasonal region #1 is not affected in the first year because the epidemic begins in this region.

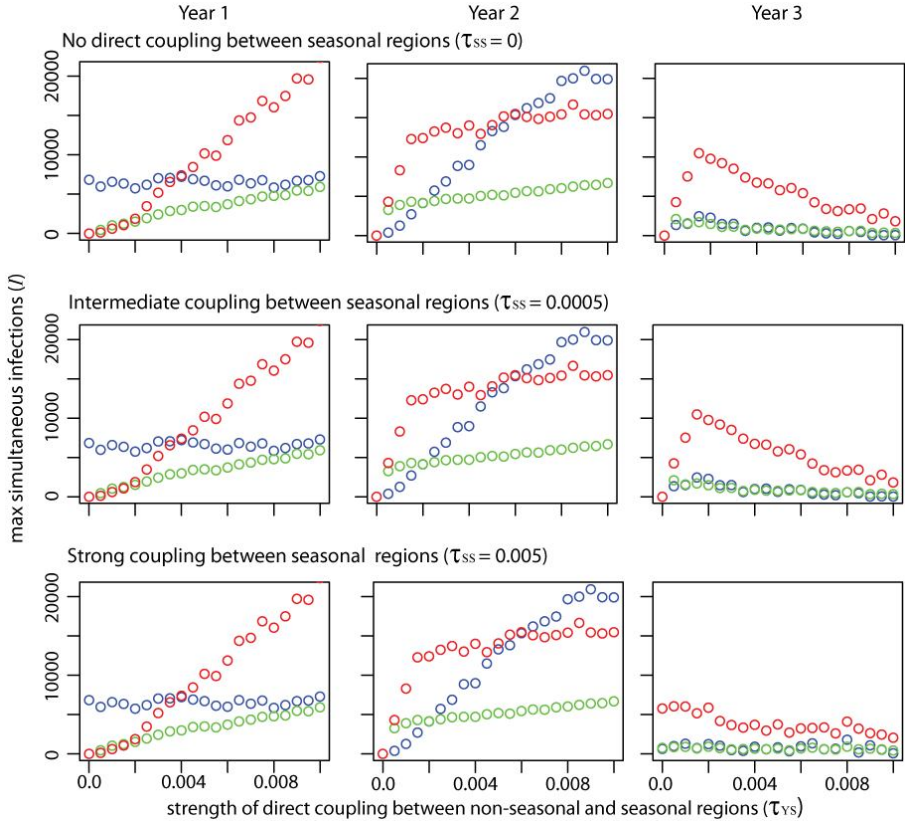


Figure S4: Epidemiological impact of coupling between seasonal and non-seasonal regions**.** Maximum number of simultaneous wildtype infections in each of the three seasons following introduction, as a function of the strength of coupling between non-seasonal and seasonal regions. Top row – no direct coupling between seasonal regions. Middle row – intermediate coupling between seasonal regions. Bottom row – strong coupling between seasonal regions. Blue: maximum number of simultaneous infections in seasonal region #1. Green: non-seasonal region. Red: seasonal region #2. Parameters and initial conditions as in Figure S3. Stronger direct coupling between seasonal and non-seasonal regions increases the size of all epidemics in the first two years. Epidemic size may decrease in the third year because immunity has accumulated more rapidly. Seasonal region #1 is not affected in the first year because the epidemic begins in this region.

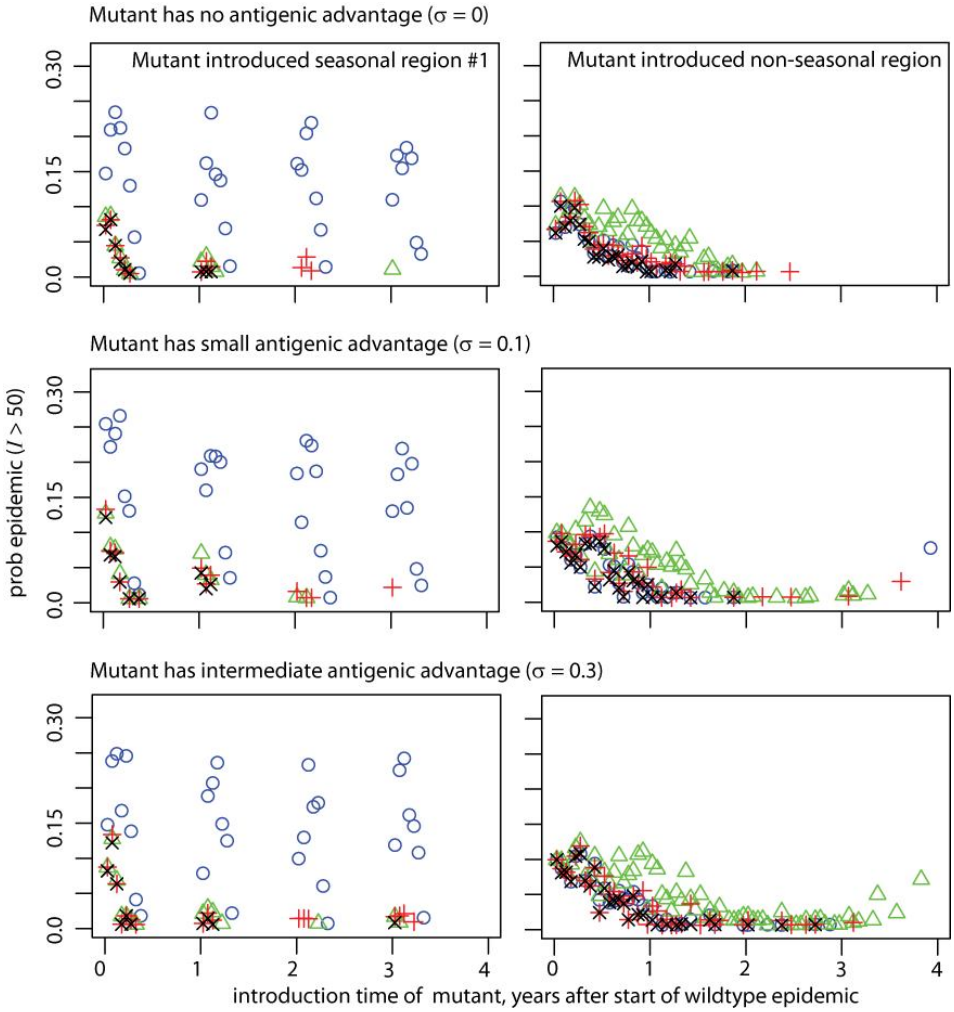


Figure S5: Epidemic potential of a single mutant depending on introduction time and antigenic advantage. Probability, as a function of introduction time relative to start of wildtype epidemic, a single individual infected with a mutant strain leads to a mutant epidemic in each region. Left panels: mutant introduced in seasonal region #1. Right panels: mutant introduced in non-seasonal region. Top row: mutant has no antigenic advantage. Middle row: mutant has small antigenic advantage. Bottom row: mutant has intermediate antigenic advantage. Blue circles: probability of mutant epidemic in seasonal region #1. Green triangles: non-seasonal region. Red pluses: seasonal region #2. Black crosses: all regions. Each panel is the result of 10000 independent model realizations in which the mutant strain is introduced at a random time during the first four years of the wildtype epidemic. Initially all populations were naive except 10 individuals infected with the wildtype strain in seasonal region #1. Parameter values as Table S1. In the seasonal region mutants have a relatively high chance of causing a local epidemic in any year if they are introduced in the early part of the transmission season. However, they are unlikely to spread to other regions unless they are introduced in the early stages of the wildtype epidemic. In the non-seasonal region, mutants have a lower, but still significant chance of causing a local epidemic, throughout the first year and half of the wildtype epidemic. During the first year of this period, mutants also have a significant, but decreasing, probability of spreading to other regions. Small to intermediate antigenic advantages have little impact on the epidemic probability of a mutant.

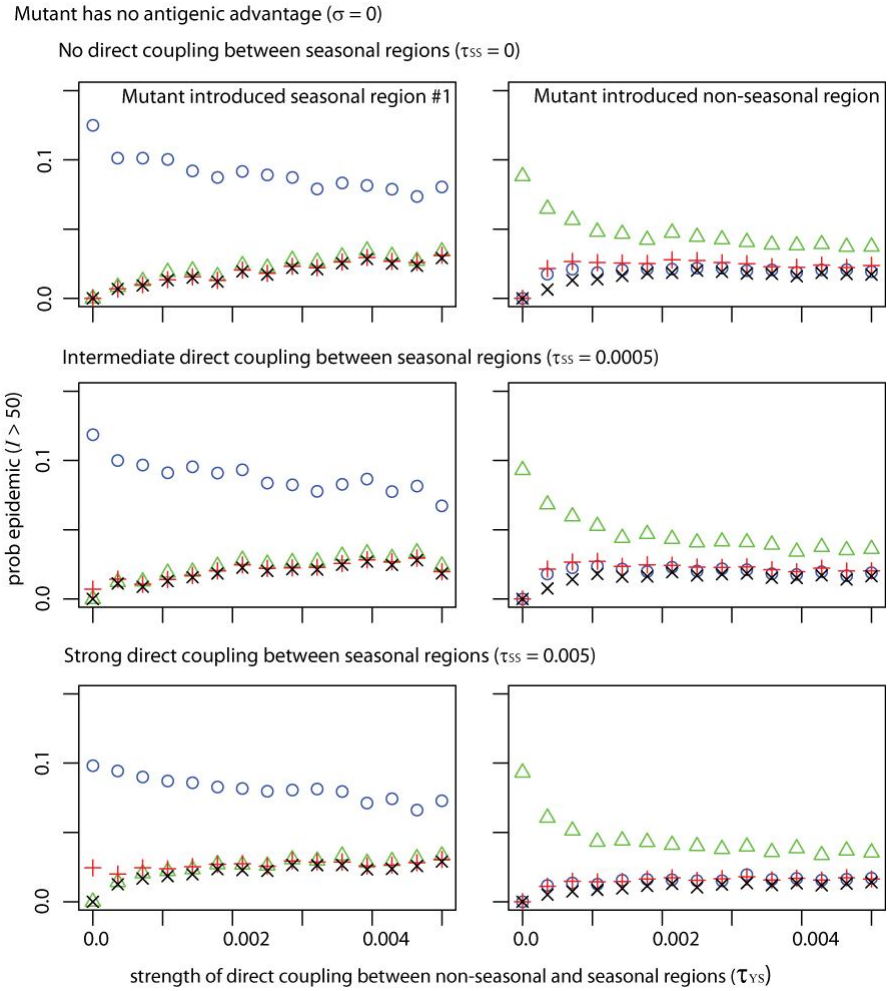


Figure S6: Epidemic potential of a single antigenically neutral mutant depending on coupling between seasonal and non-seasonal regions. Probability that the random introduction of a single individual infected with a mutant strain carrying no antigenic advantage leads to a mutant strain epidemic in each region. Left panels: mutant strain introduced to seasonal region #1. Right panels: mutant strain introduced to non-seasonal region. Top row: no direct coupling between seasonal regions. Middle row: intermediate direct coupling between seasonal regions. Bottom row: strong direct coupling between seasonal regions. Blue circles: probability of mutant epidemic in seasonal region #1. Green triangles: non-seasonal region. Red pluses: seasonal region #2. Black crosses: all regions. An epidemic is defined as at least 50 simultaneous infections. The model is composed of all three regions, a wildtype virus strain and a single mutant strain with no antigenic advantage relative to the wildtype. Each point is the result of 10000 independent trials in which the mutant strain is introduced at a random time during the first four years of the wildtype epidemic. Initially all populations were naive except 10 individuals infected with the wildtype strain in seasonal region #1. Parameter values as Table S1. Stronger coupling between seasonal and non-seasonal regions increases the probability that mutants in seasonal regions will spread to other regions. Stronger coupling also initially increases the probability that mutants in non-seasonal regions will spread, but the impact saturates for higher coupling.

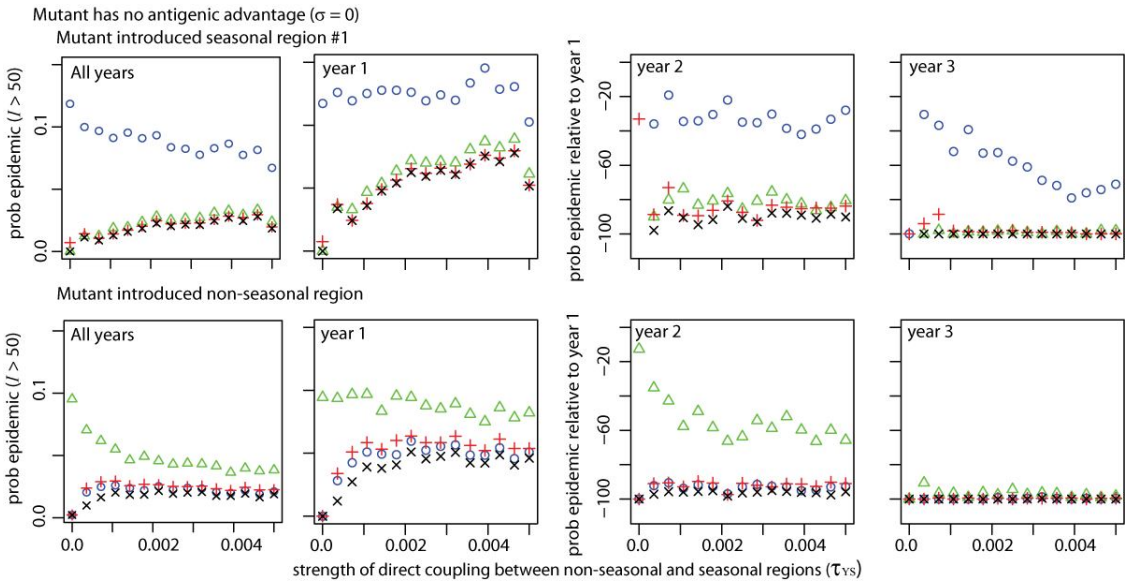


Figure S7: Epidemic potential of a single antigenically neutral mutant depending on coupling between seasonal and non-seasonal regions and the year in which it is introduced. Left panel: probability that a single individual infected with a mutant strain introduced at a random time in the first four years of the wildtype epidemic leads to a mutant epidemic in each region. Centre left panel: epidemic probability if the mutant strain is introduced in year 1. Centre right and right panels: epidemic probability if the mutant is introduced in year 2, or year 3, expressed as the percentage change relative to year 1. Top row: mutant strain introduced to seasonal region #1. Bottom row: mutant strain introduced to non-seasonal region. Blue circles: probability of mutant epidemic in seasonal region #1. Green triangles: non-seasonal region. Red pluses: seasonal region #2. Black crosses: all regions. Each point is the result of 10000 independent trials in which the mutant strain is introduced at a random time during the first four years of the wildtype epidemic. Initial conditions and parameter values as in Figure S6. Stronger coupling between seasonal and non-seasonal regions increases the probability that mutants in seasonal regions will spread to other regions. Stronger coupling also initially increases the probability that mutants in non-seasonal regions will spread, but the impact saturates for higher coupling. Stronger coupling has a similar impact on mutants in the second year, but the epidemic probability is considerably lower for mutants introduced in the second year, and almost zero in the third year.

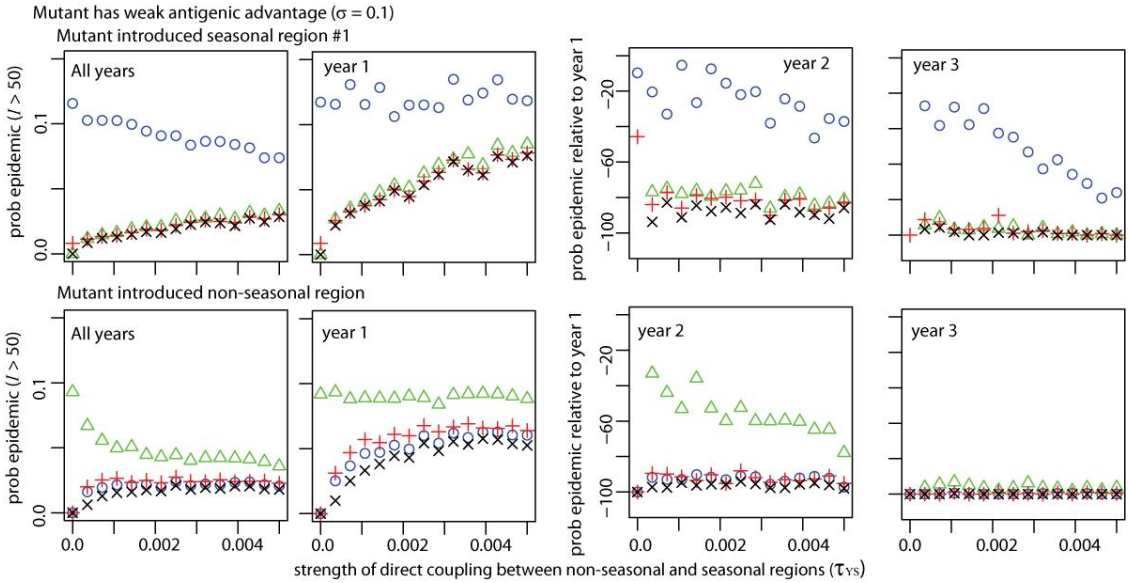


Figure S8: Epidemic potential of a single mutant with a small antigenic advantage relative to the wildtype strain depending on coupling between seasonal and non-seasonal regions and the year in which it is introduced. Left panel: probability that a single individual infected with a mutant strain introduced at a random time in the first four years of the wildtype epidemic leads to a mutant epidemic in each region. Centre left panel: epidemic probability if the mutant strain is introduced in year 1. Centre right and right panels: epidemic probability if the mutant is introduced in year 2, or year 3, expressed as the percentage change relative to year 1. Top row: mutant strain introduced to seasonal region #1. Bottom row: mutant strain introduced to non-seasonal region. Blue circles: probability of mutant epidemic in seasonal region #1. Green triangles: non-seasonal region. Red pluses: seasonal region #2. Black crosses: all regions. Each point is the result of 10000 independent trials in which the mutant strain is introduced at a random time during the first four years of the wildtype epidemic. Initial conditions and parameter values as in Figure S6. For a mutant with a weak antigenic advantage, the relationship between the epidemic probability and the strength of coupling is very similar to that for a neutral mutant shown in Figure S7.

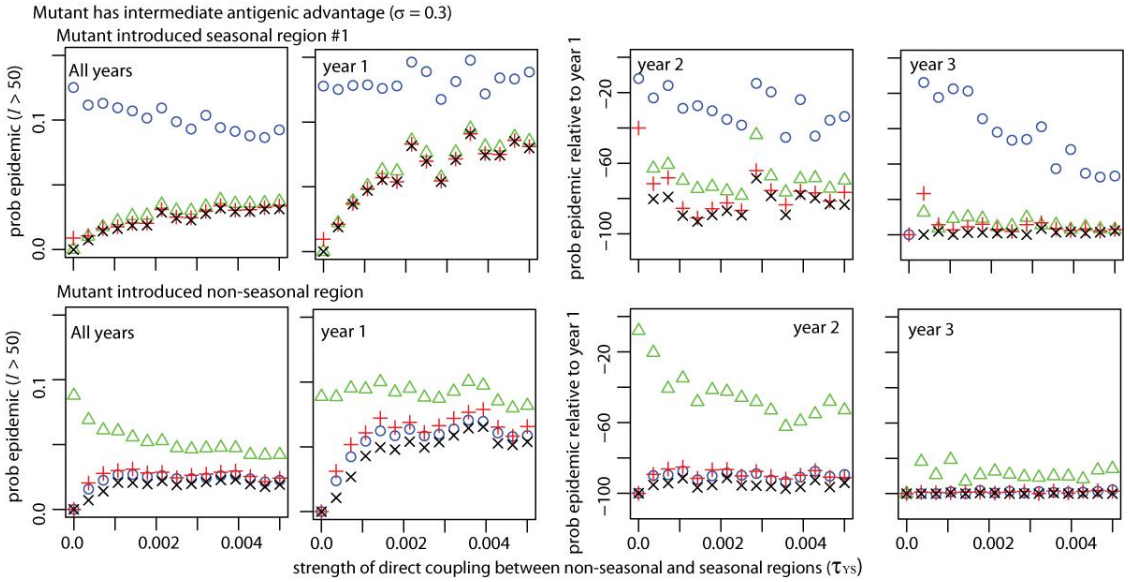


Figure S9: Epidemic potential of a single mutant with an intermediate antigenic advantage relative to the wildtype strain depending on coupling between seasonal and non-seasonal regions and the year in which it is introduced. Left panel: probability that a single individual infected with a mutant strain introduced at a random time in the first four years of the wildtype epidemic leads to a mutant epidemic in each region. Centre left panel: epidemic probability if the mutant strain is introduced in year 1. Centre right and right panels: epidemic probability if the mutant is introduced in year 2, or year 3, expressed as the percentage change relative to year 1. Top row: mutant strain introduced to seasonal region #1. Bottom row: mutant strain introduced to non-seasonal region. Blue circles: probability of mutant epidemic in seasonal region #1. Green triangles: non-seasonal region. Red pluses: seasonal region #2. Black crosses: all regions. Each point is the result of 10000 independent trials in which the mutant strain is introduced at a random time during the first four years of the wildtype epidemic. Initial conditions and parameter values as in Figure S6. For a mutant with an intermediate antigenic advantage, the relationship between the epidemic probability and the strength of coupling is very similar to that for a neutral mutant shown in Figure S7.

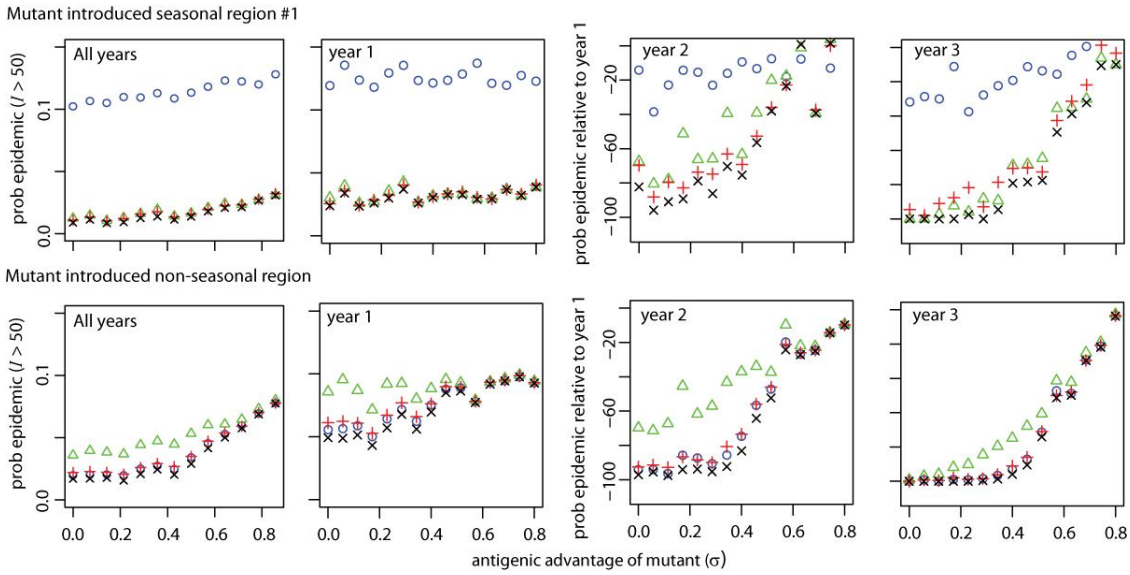


Figure S10: Epidemic potential of a single mutant depending on year of introduction and antigenic advantage. Left panel: Probability, depending on antigenic advantage of mutant relative to wildtype strain, that a single individual infected with a mutant strain introduced at a random time in the first four years of the wildtype epidemic leads to a mutant epidemic in each region. Centre left panel: epidemic probability if the mutant strain is introduced in year 1. Centre right and right panels: epidemic probability if the mutant is introduced in year 2, or year 3, expressed as the percentage change relative to year 1. Top row: mutant strain introduced to seasonal region #1. Bottom row: mutant strain introduced to non-seasonal region. Blue circles: probability of mutant epidemic in seasonal region #1. Green triangles: non-seasonal region. Red pluses: seasonal region #2. Black crosses: all regions. Each point is the result of 10000 independent trials in which the mutant strain is introduced at a random time during the first four years of the wildtype epidemic. Initial conditions and parameter values as in Figure S6. Mutants with a greater antigenic advantage are more likely to lead to local epidemics, and spread to other regions. However, the epidemic probability only begins to increase significantly when the antigenic advantage exceeds 0.5. The impact of the antigenic advantage is most pronounced in the non-seasonal region, in the second and third years, when immunity has accumulated due to the wildtype epidemic.

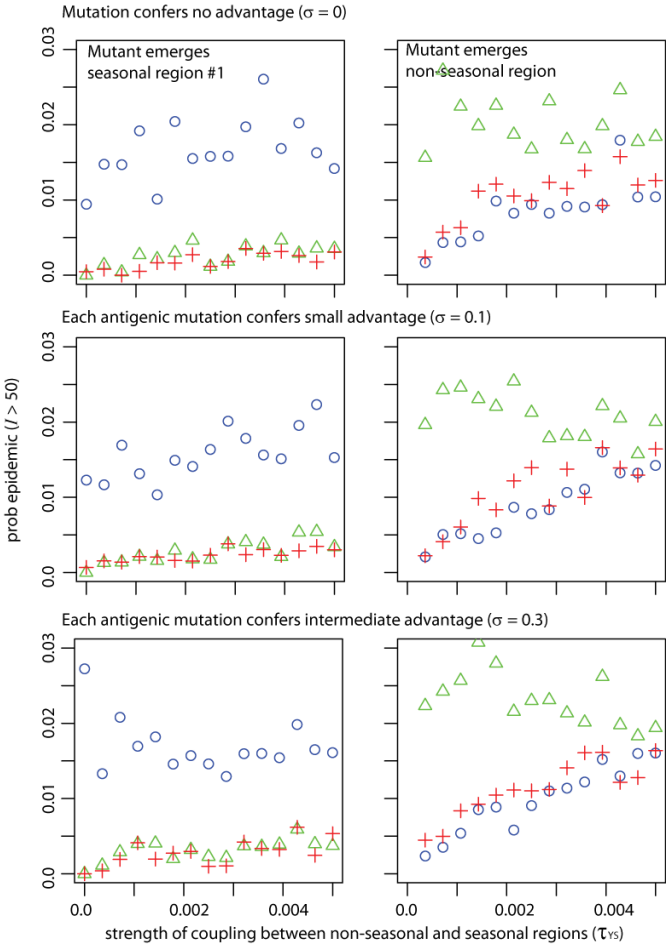


Figure S11: Epidemic potential of spontaneous mutants depending on coupling between seasonal and non-seasonal regions and the advantage conferred by each antigenic mutation. Probability that a novel strain emerging by spontaneous mutation of an existing strain causes an epidemic in each region as a function of the strength of coupling between seasonal and non-seasonal regions. Left panels: mutants that emerge in seasonal region #1. Right panels: mutants that emerge in non-seasonal region. Top row: all mutations are antigenically neutral. Middle row: each antigenic mutation confers a small advantage (0.1 re-infection probability for each difference between antigenic genotype sections). Bottom row: each antigenic mutation confers an intermediate advantage (0.3 re-infection probability for each difference between antigenic genotype sections). Blue circles: probability of mutant epidemic in seasonal region #1. Green triangles: non-seasonal region. Red pluses: seasonal region #2. An epidemic is defined as at least 50 simultaneous infections. The model is composed of all three regions, a wildtype virus strain and any number of mutant strains emerging from the wildtype strain, or other mutants. Each point is the result of 100 independent realizations of the model. Initially all populations were naive except 10 individuals infected with the wildtype strain in seasonal region #1. Parameter values as Table S1. Stronger coupling between seasonal and non-seasonal regions leads to a small increase in the probability that spontaneous mutants in seasonal regions will spread to other regions. Stronger coupling also leads to consistently increasing probabilities that spontaneous mutants in non-seasonal regions will spread to other regions.

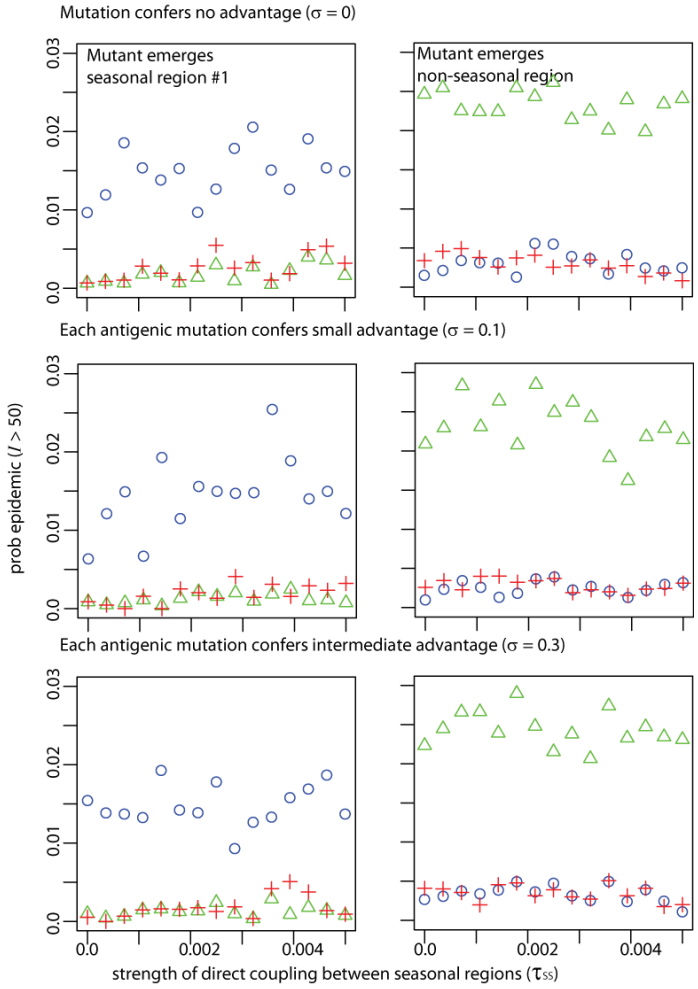


Figure S12: Epidemic potential of spontaneous mutants depending on direct coupling between seasonal regions and advantage of each antigenic mutation. Probability that a novel strain emerging by spontaneous mutation of an existing strain causes an epidemic in each region as a function of the strength of direct coupling between seasonal regions. Left panels: mutants that emerge in seasonal region #1. Right panels: mutants that emerge in non-seasonal region. Top row: all mutations are antigenically neutral. Middle row: each antigenic mutation confers a small advantage (0.1 re-infection probability for each difference between antigenic genotype sections). Bottom row: each antigenic mutation confers an intermediate advantage (0.3 re-infection probability for each difference between antigenic genotype sections). Blue circles: probability of mutant epidemic in seasonal region #1. Green triangles: non-seasonal region. Red pluses: seasonal region #2. Each point is the result of 100 independent realizations. Initial conditions and parameter values as in Figure S11. Stronger direct coupling between seasonal regions has little impact on the probability that spontaneous mutants cause local or global epidemics.

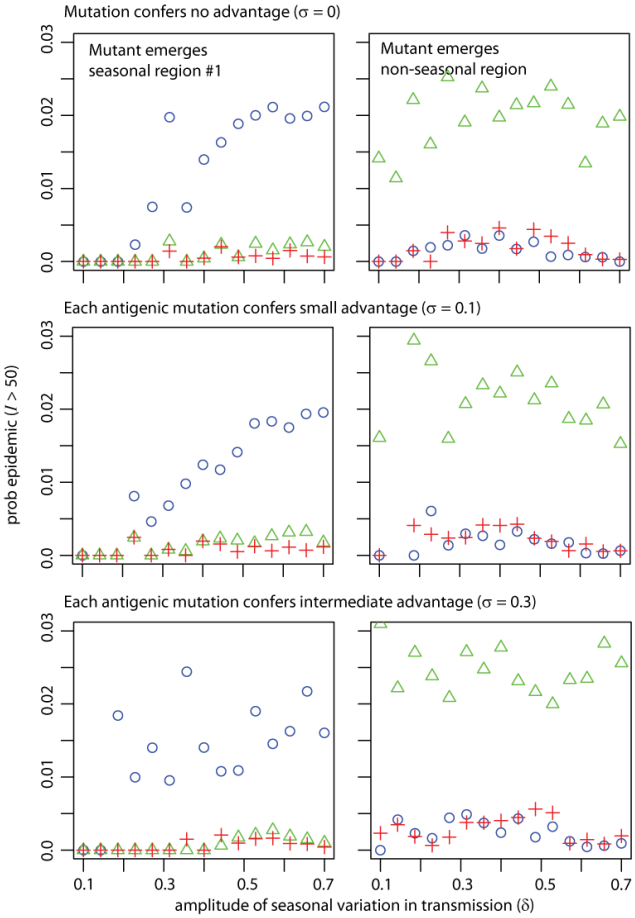


Figure S13: Epidemic potential of spontaneous mutants depending on amplitude of seasonal variation in transmission intensity and advantage of each antigenic mutation. Probability that a novel strain emerging by spontaneous mutation of an existing strain causes an epidemic in each region as a function of the amplitude of seasonal variation in transmission intensity. Left panels: mutants that emerge in seasonal region #1. Right panels: mutants that emerge in non-seasonal region. Top row: all mutations are antigenically neutral. Middle row: each antigenic mutation confers a small advantage (0.1 re-infection probability for each difference between antigenic genotype sections). Bottom row: each antigenic mutation confers an intermediate advantage (0.3 re-infection probability for each difference between antigenic genotype sections). Blue circles: probability of mutant epidemic in seasonal region #1. Green triangles: non-seasonal region. Red pluses: seasonal region #2. Each point is the result of 100 independent realizations. Initial conditions and parameter values as in Figure S11. When the amplitude is low, seasonal transmission intensity is too small for sustained outbreak. As the amplitude becomes larger, sustained transmission become possible, and mutants can cause epidemics in the seasonal region. There is no clearly defined impact on the epidemic potential of non-seasonal mutants.

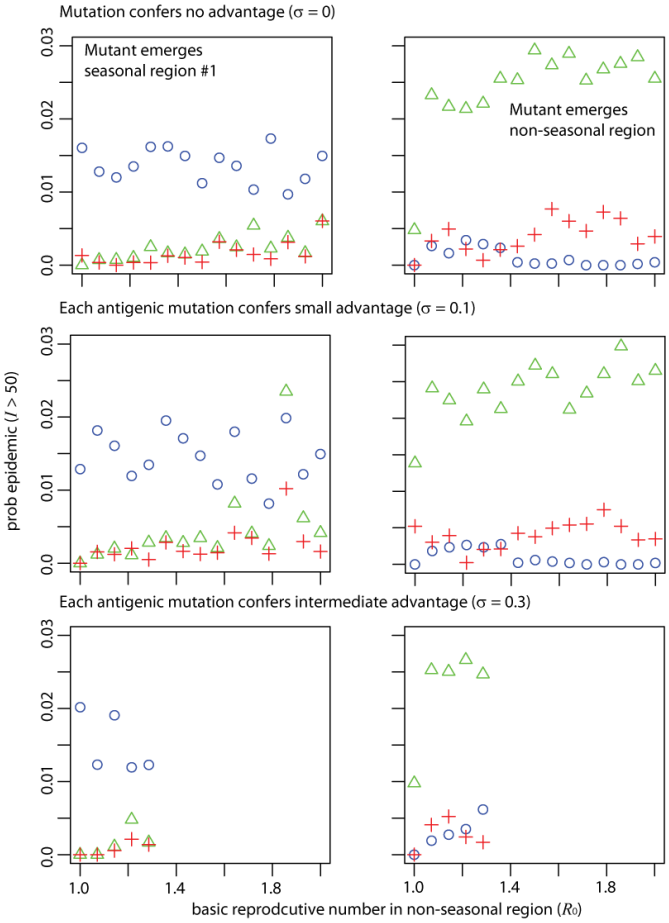


Figure S14: Epidemic potential of spontaneous mutants depending on transmission intensity the in non-seasonal region and the advantage of each antigenic mutation. Probability that a novel strain emerging by spontaneous mutation of an existing strain causes an epidemic in each region as a function of the transmission intensity in the non-seasonal regions, expressed in terms of the basic reproductive number $R_0 = \beta N/(\gamma + \mu)$. Left panels: mutants that emerge in seasonal region #1. Right panels: mutants that emerge in non-seasonal region. Top row: all mutations are antigenically neutral. Middle row: each antigenic mutation confers a small advantage (0.1 re-infection probability for each difference between antigenic genotype sections). Bottom row: each antigenic mutation confers an intermediate advantage (0.3 re-infection probability for each difference between antigenic genotype sections). For this intermediate antigenic advantage, computation became impractically slow for $R_0 > 1.3$ because of an explosion of diversity in some realizations. Blue circles: probability of mutant epidemic in seasonal region #1. Green triangles: non-seasonal region. Red pluses: seasonal region #2. Each point is the result of 100 independent realizations. Initial conditions and parameter values as in Figure S11. More intense transmission in the non-seasonal region increases the probability that mutants arising in that region cause local epidemics. It may also increase the probability that they spread to seasonal regions, but the pattern is not clear, and may be complicated by the impact the change in non-seasonal transmission has on the basic epidemiology of all regions. If each mutation confers a small antigenic advantage the trend is similar. If mutation confers an intermediate antigenic advantage, the impact of increasing non-seasonal transmission intensity is more pronounced and there is a critical transmission threshold at which there is an explosion of diversity.
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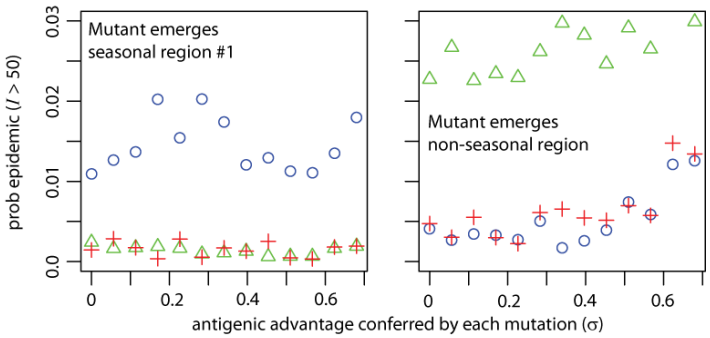


Figure S15: Epidemic potential of spontaneous mutants depending on the advantage of antigenic mutations. Probability that a novel strain emerging by spontaneous mutation of an existing strain causes an epidemic in each region as a function of the antigenic advantage associated with each mutation, where the re-infection probability is σ for each difference between antigenic genotype sections. Left panels: mutants that emerge in seasonal region #1. Right panels: mutants that emerge in non-seasonal region. Blue circles: probability of mutant epidemic in seasonal region #1. Green triangles: non-seasonal region. Red pluses: seasonal region #2. Each point is the result of 100 independent realizations. Initial conditions and parameter values as in Figure S11. A greater antigenic advantage associated with each mutation leads to a small increase in the epidemic probability. However, if the antigenic advantage exceeds a certain threshold, in this case around 0.7 there may be an explosive increase in diversity.

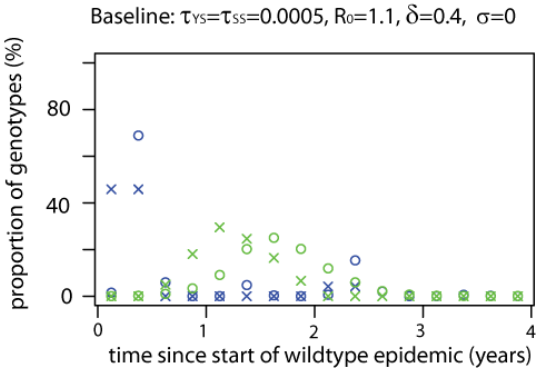


Figure S16: Distribution of emergence times of all spontaneous mutants, and those spontaneous mutants that cause significant outbreaks. Baseline case, all mutations antigenically neutral. Circles: distribution of emergence times of spontaneous mutants, expressed as the proportion of all the mutants appearing over the first four years of the wildtype epidemic that emerge in the given time interval. Crosses: distribution of emergence times of successful mutants, expressed as the proportion of all mutants that cause significant outbreaks appearing over the first four years that emerge in the given time interval. Blue: mutants emerging in seasonal region #1. Green: mutants emerging in non-seasonal region. The model is composed of all three regions, a wildtype strain and any number of mutant strains emerging from the wildtype strain, or other mutants. The figure summarizes 100 independent realizations of the model. Initially all populations were naive except 10 individuals infected with the wildtype strain in seasonal region #1. Parameter values as inTable S1. In the seasonal region the majority of mutants appear in the second half of the first year epidemic. The majority of successful mutants appear in the first year epidemic, notably in the first half of this epidemic. In the non-seasonal region the majority of mutants appear after one and a half to two years. The majority of successful mutants appear after around one year.

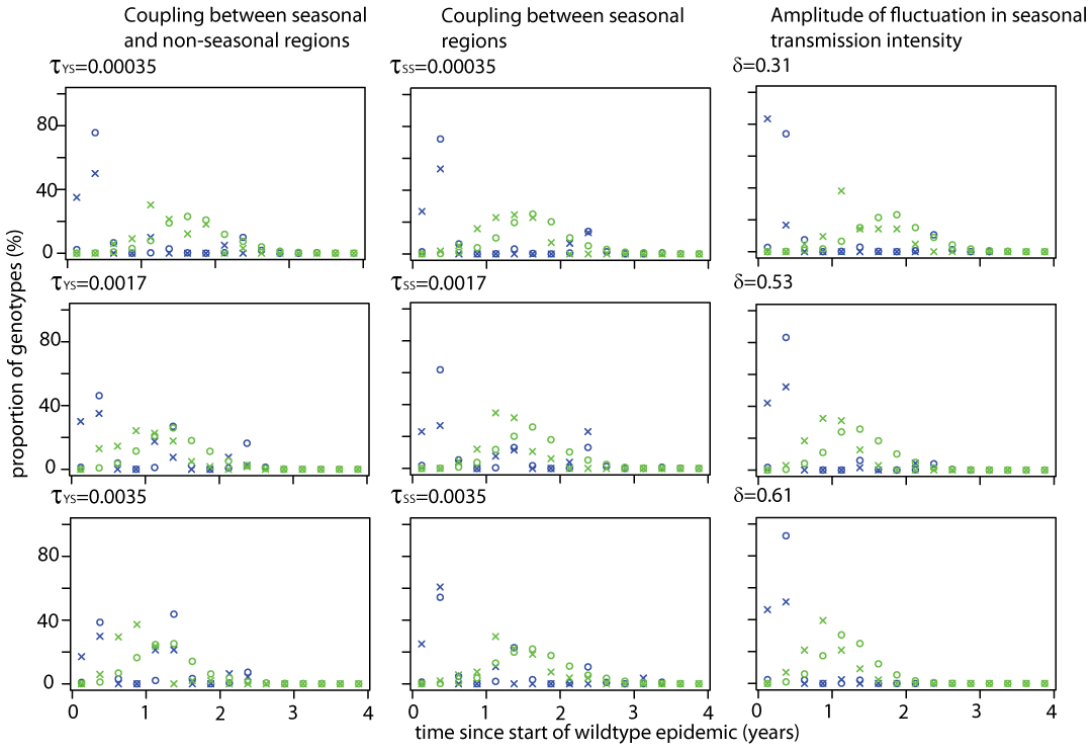


Figure S17: Distribution of emergence times of all spontaneous mutants, and those spontaneous mutants that cause significant outbreaks, depending on coupling between regions. All mutations antigenically neutral. Left column: strength of coupling between seasonal and non-seasonal regions. Centre column: strength of coupling between seasonal regions. Right column: amplitude of fluctuations in seasonal transmission intensity. Circles: distribution of emergence times of spontaneous mutants, expressed as the proportion of all the mutants appearing over the first four years of the wildtype epidemic that emerge in the given time interval. Crosses: distribution of emergence times of successful mutants, expressed as the proportion of all mutants that cause significant outbreaks appearing over the first four years that emerge in the given time interval. Blue: mutants emerging in seasonal region #1. Green: mutants emerging in non-seasonal region. Each panel is based on 100 independent realizations of the model. Initial conditions and parameter values as in Figure S15. Stronger coupling between seasonal and non-seasonal regions causes a greater proportion of all mutants, and successful mutants, to appear in the second year. However, in all years, the successful mutants generally appear before the majority. Stronger coupling between seasonal and non-seasonal regions causes the epidemic to peak, and produce the majority of mutants, earlier. But the majority of successful mutants still emerge before this peak. The strength of direct coupling between seasonal regions has little impact. Higher maximum intensity transmission in the seasonal region also accelerates the epidemiology, resulting in the majority of non-seasonal mutants appearing earlier, and the majority of seasonal mutants appearing in the first year.

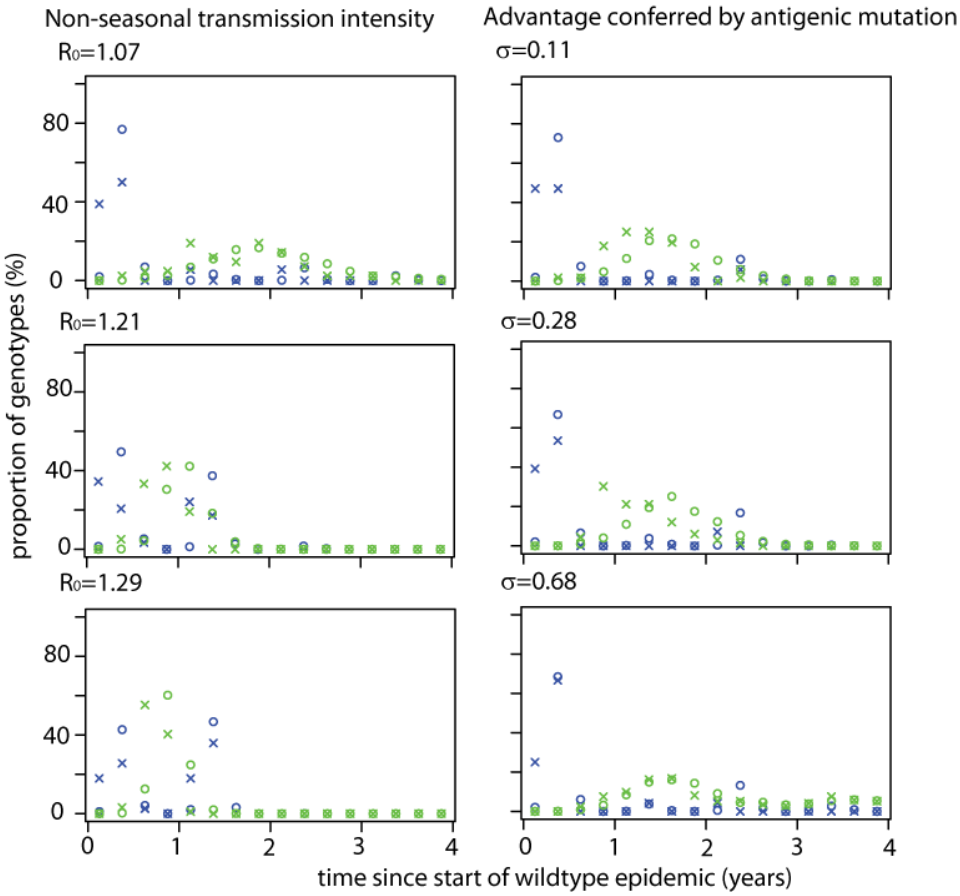


Figure S18: Distribution of emergence times of all spontaneous mutants, and those spontaneous mutants that cause significant outbreaks, depending on seasonal and non-seasonal transmission intensity. All mutations antigenically neutral. Left column: non-seasonal transmission intensity, expressed in terms of $R_0 = \beta N/(\gamma + \mu)$. Right column: advantage conferred by each antigenic mutation. Circles: distribution of emergence times of spontaneous mutants, expressed as the proportion of all the mutants appearing over the first four years of the wildtype epidemic that emerge in the given time interval. Crosses: distribution of emergence times of successful mutants, expressed as the proportion of all mutants that cause significant outbreaks appearing over the first four years that emerge in the given time interval. Blue: mutants emerging in seasonal region #1. Green: mutants emerging in non-seasonal region. Each panel is based on 100 independent realizations of the model. Initial conditions and parameter values as in Figure S15. Higher intensity transmission in the non-seasonal region accelerates the epidemiology, resulting in the majority of mutants in the non-seasonal region appearing earlier, and mutants in the seasonal regions being spread more evenly between the first and second years. Weak to intermediate advantages associated with antigenic mutations have little impact. A strong advantage leads to a more even distribution of emergence times of mutants in the non-seasonal region and a closely corresponding distribution for the emergence times of successful mutants. Mutants that appear earlier than the majority are no longer favoured.

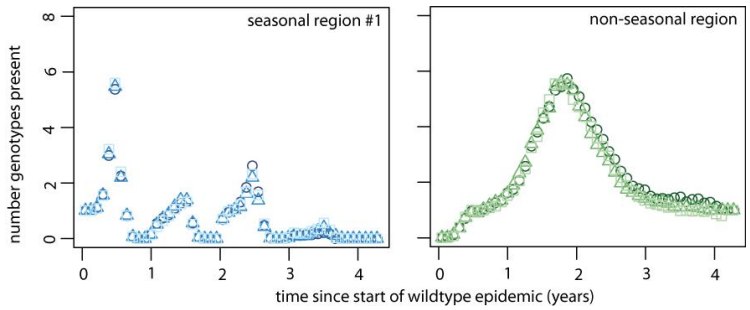


Figure S19: Transient diversity of the virus population depending on time since the start of the wildtype epidemic. Left: number of distinct genotypes present in seasonal region #1. Right: number of distinct genotypes present in non-seasonal region at time *t*. Palest squares: each mutation has no antigenic advantage. Triangles: small antigenic advantage ($\sigma = 0.1$). Dark circles: intermediate advantage ($\sigma = 0.3$). Based on 100 independent realizations of the model. Initial conditions and parameter values as in Figure S11. Diversity is highest around the epidemic peaks, when new mutants are being rapidly generated. Small or intermediate antigenic advantages associated with mutation do not have a significant impact on diversity.

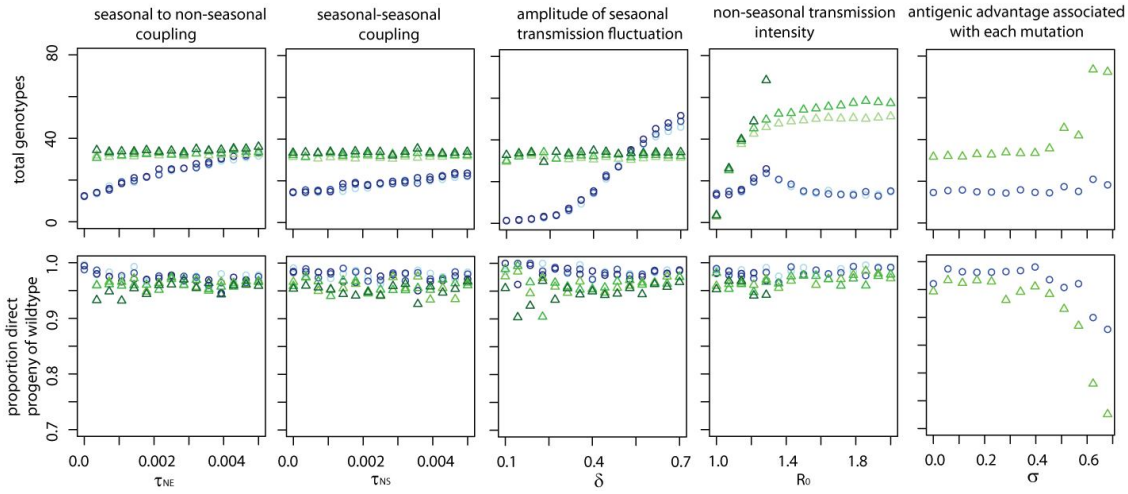


Figure S20: Cumulative genetic diversity of the virus population. Top row: total number of distinct genotypes detected in each region over the course of an epidemic lasting up to 8 years when new strains emerge by spontaneous mutation of existing strains. Bottom row: proportion of these genotypes that only differ from the wildtype at one antigenic genotype location. Blue circles – seasonal region #1, green triangles – non-seasonal region. In all but the final column, pale shades indicate that mutation has no antigenic advantage, intermediate shades indicate that mutation has a weak antigenic advantage ($\sigma = 0.1$) and dark shades indicate an intermediate advantage ($\sigma = 0.3$). Each point is the mean of 100 independent realizations of the model. Initial conditions and parameter values as in Figure S11. The strength of coupling between regions has little impact on diversity. Increasing the amplitude of the variation in seasonal transmission intensity increases diversity in the seasonal region if the effective reproductive number is close to 1. Increasing transmission intensity in the non-seasonal region increases diversity in the seasonal region, but has little impact on the non-seasonal region. If each mutation confers an intermediate antigenic advantage, increasing non-seasonal transmission intensity may lead to an explosive increase diversity. For $\sigma = 0.3$, this explosion occurs when $R_0 = 1.3$ and made further computation unfeasible. Similarly, for fixed $R_0$, increasing the antigenic advantage associated with each mutation has little impact on diversity until a critical threshold is reached, here around 0.7, beyond which there is an explosive increase in diversity and further computation becomes unfeasible.

**Detailed Model Description**

**Epidemiological model**

We use a standard SIR framework (1-5) for multiple co-circulating strains with immune cross-reaction. We divide the host population into seasonal regions $S_1$ and $S_2$, and a non-seasonal (year-round) region $Y$. In each region we group hosts according to their current immune state and infectious state, as set out below. To incorporate immune cross-reaction we let $P$ be the set of all possible antigenically differentiable genotypes. Let $A$ be any subset of $P$, including $P$ and Ø. Let $i$ be a single member of $P$. We then make the following assumptions:

- A host can be infected by at most one strain at a time. This assumption is reasonable since infected hosts are likely to have much less contact with the rest of the population. They may also have a heightened immune state. The observation of reassortment (6) indicates that multiple simultaneous infections do occur but we assume this is sufficiently rare to ignore for the purposes of this model.
- Cross-immunity reduces susceptibility. Previous infection with the set of strains $A$ reduces the probability that a challenge by strain $i$ is successful by a factor $f(A, i)$. If $f(A, i) = 0$ then protection is perfect and infection is impossible. If $f(A, i) = 1$ then protection is absent. The exact form of $f$ is specified below.
- The host population in each region is well mixed. Each host has an equal probability of meeting any other host in the same region.
- The contact rate $\beta(t)$ is the expected number of host encounters sufficient for transmission per unit time. In year-round regions this is constant, $\beta(t) = \beta$. In seasonal regions it varies throughout the year: $\beta(t) = \beta_0(1 + \delta\sin(2\pi(t + \psi))$ for $0 < t < 0.5$ and $\beta(t) = 0$ for $0.5 < t < 1$. Here $\psi$ is the offset. For seasonal region #1 $\psi = 0$. For seasonal region #2 $\psi = 0.5$, making the transmission periods exactly out of phase.
- The contact rate between hosts is scaled by a factor $\tau_{ZW}$ according to the regions $W$ and $Z$ in which they are located. Within all regions the contact rate is unchanged: $\tau_{ZZ} = 1$. Between regions the contact rate is greatly reduced: $\tau_{ZW} << 1$.
- The birth rate of each individual in the population is $\mu$ per year. The death rate is also $\mu$. So the total population size remains constant. All new births are fully susceptible to all virus strains.
- Infected hosts recover after an average of $1/\gamma$ years.

We group the host population into classes according to the set of antigenically differentiable strains they have previously experienced and the strain with which they are currently infected. Let $R^Z_A$ be the set of all hosts in region $Z$ that have previously recovered from infection with all the strains in set $A$. The set $R^Z_\emptyset$ contains hosts that have never been infected. Let $I^Z_{A,i}$ be the set of all hosts that are currently infected with strain $i$ and have previously recovered from infection with all the strains in set $A$. All hosts fall into exactly one of these sets. If the number of hosts in each set is considered continuous, and there is no mutation, differential equations describing the epidemiological dynamics in each region $Z = S_1, S_2, Y$ are:

$$\frac{dR^Z_\varnothing}{dt} = \mu \sum_{X \subseteq P} \left( R^Z_X + \sum_{i \in P} I^Z_{X,i} \right) - \beta \left( \sum_{i \in P} \sum_{X \subseteq P} I^Z_{X,i} \right) R^Z_\varnothing - \mu R^Z_\varnothing$$

$$\frac{dR^Z_A}{dt} = \gamma \sum_{i \in P} I^Z_{A/i,i} - \beta \left( \sum_{i \in P} f(A,i) \sum_{X \subseteq P} I^Z_{X,i} \right) R^Z_A - \mu R^Z_A$$

$$\frac{dI^Z_{A,i}}{dt} = \beta f(A,i) \left( \sum_{X \subseteq P} I^Z_{X,i} \right) R^Z_A - (\gamma + \mu) I^Z_{X,i}$$

In order to couple regions these equations are modified. Hosts in region $Z$ are also exposed to a small proportion of the infected hosts in the two other regions $V, W \neq Z$ (7). The equations become:

$$\frac{dR^Z_\varnothing}{dt} = \mu \sum_{X \subseteq P} \left( R^Z_X + \sum_{i \in P} I^Z_{X,i} \right) - \beta \left( \sum_{i \in P} \left[ \sum_{X \subseteq P} I^Z_{X,i} + \tau_{ZW} \sum_{X \subseteq P} I^W_{X,i} + \tau_{ZV} \sum_{X \subseteq P} I^V_{X,i} \right] \right) R^Z_\varnothing - \mu R^Z_\varnothing$$

$$\frac{dR^Z_A}{dt} = \gamma \sum_{i \in P} I^Z_{A/i,i} - \beta \left( \sum_{i \in P} f(A,i) \left[ \sum_{X \subseteq P} I^Z_{X,i} + \tau_{ZW} \sum_{X \subseteq P} I^W_{X,i} + \tau_{ZV} \sum_{X \subseteq P} I^V_{X,i} \right] \right) R^Z_A - \mu R^Z_A$$

$$\frac{dI^Z_{A,i}}{dt} = \beta f(A,i) \left( \left[ \sum_{X \subseteq P} I^Z_{X,i} + \tau_{ZW} \sum_{X \subseteq P} I^W_{X,i} + \tau_{ZV} \sum_{X \subseteq P} I^V_{X,i} \right] \right) R^Z_A - (\gamma + \mu) I^Z_{X,i}$$

This deterministic expression of the system can be transformed into a stochastic model with a continuous time variable and discrete population variables by expressing it in terms of the probability of each possible event (8-9):

| Event | Change | Probability |
|---|---|---|
| Host in immune class $R^Z_A$ challenged by host in infected class $I^W_{X,i}$ | None | $\tau_{ZW}\beta(t)R^Z_A I^Z_{X,i}$ |
| Above challenge successful | $R^Z_A \rightarrow R^Z_A - 1$, $I^Z_{A,i} \rightarrow I^Z_{A,i} + 1$ | $f(A,i)$ |
| Host in infected class $I^Z_{A,i}$ recovers | $I^Z_{A,i} \rightarrow I^Z_{A,i} - 1$ $R^Z_{A \cup i} \rightarrow R^Z_{A \cup i} + 1$ | $\gamma I^Z_{A,i}$ |
| Host in immune class $R^Z_A$ dies and replaced by new birth | $R^Z_A \rightarrow R^Z_A - 1$ $R^Z_\varnothing \rightarrow R^Z_\varnothing + 1$ | $\mu R^Z_A$ |
| Host in infected class $I^Z_{A,i}$ dies and replaced by new birth | $I^Z_{A,i} \rightarrow I^Z_{A,i} - 1$ $R^Z_\varnothing \rightarrow R^Z_\varnothing + 1$ | $\mu I^Z_{A,i}$ |

If there are $p$ elements in $P$ then the number of possible subsets $A$ is $2^p$. We need to keep track of all possible immune classes, and the combination of each of these classes with an infection strain. So the model requires $(p + 1)2^p$ variables. It also requires a set of transition equations for each variable. This system is enormous even when $p$ is small. However, most of these variables will be 0 because at least one of the strains in the subset $A$ has not yet existed or the classes $R^Z_A$ and $I^Z_{A,i}$ do exist but have become empty due to further infections or recovery. Classes with 0 members have no impact on the model. They need not be considered. Computationally, they can be created as needed and destroyed when no longer needed. This greatly reduces the number of active variables and transitions.

Other methods have been used to get round the problems of such a large system. The most feasible assumes that multiple simultaneous infections can occur, immunity reduces infectivity rather than susceptibility and immunity is polarized. After infection a host either gains complete immunity with probability $f(A, i)$ or no immunity with probability $1 - f(A, i)$ (10-12). All hosts are always susceptible to all strains but not all hosts transmit all strains. Since all host are susceptible immunity can accumulate more quickly than in models where susceptibility is reduced and re-infection is rarer. This process may be thought of as a form of boosting. It is powerful because, during a large epidemic, a host may be re-infected multiple times. It will have multiple opportunities to gain immunity to all strains cross-reactive with the infecting strain. The great advantage of this model framework is the host population can be grouped into just $2p$ overlapping classes for each region $S^Z_i$ – susceptible to strain $i$, and $I^Z_i$ – infected with strain $i$. An alternative is to assume that the immune cross-reaction is the same between all strains (13-14). The host population can then be grouped according to whether or not they have been previously infected with any strain. Finally, the system can be simplified by assuming that immunity is polarized but acts on susceptibility. The size of higher order immune groups (those that have previously experienced more than two strains) can be approximated in terms of lower order groups (15). This approximation has been shown to be reasonably accurate for a four strain system but its impact in a system with many strains is not clear.

**Evolutionary model**

In describing the epidemiological model we defined $i$ to be an antigenically differentiable strain and $A$ to be a set of such strains. In order to model mutation and immune interaction an explicit representation of each strain $i$ is required. This encoding is achieved using a binary string. The string is divided into two sections. Section $G_a$, of length $m_a$, corresponds to antigenic sites and determines the antigenic phenotype. Section $G_n$, of length $m_n$ corresponds to sites that are neutral with respect to the antigenic phenotype. Only the antigenic section of the genotype has an impact on the epidemiological population dynamics. The neutral section is just towed along. Each element of the bitstring may be thought of as a locus, with two possible allelic states (5, 16). Alternatively each element may be thought of as an amino acid or nucleotide, again abstracted as having two possible states (17). Encodings closer to those observed in reality could also be used (18).

As long as a host is infected with strain $i$, each element of the bitstring genotype for $i$ switches with at a very low rate $\eta$ per year, creating a new strain $j$. Note that a host only gains immunity to a strain on recovery. In this case the host gains immunity to strain $j$, not strain $i$. For each infected host $I_{A,i}$ the antigenic section of the genotype defines $i$. The neutral section plays no part in the epidemiological dynamics and so does not appear in that system. However, for each class $I_{A,i}$ a list of all the neutral genotypes sections currently associated with infections in this class is maintained, together with the frequency of each of them. The following events are added to the stochastic model:

| Event | Change | Probability |
|---|---|---|
| Antigenic mutation in infected host set $I^Z_{A,i}$, strain $i$ switches to strain $j$ | $I^Z_{A,i} \rightarrow I^Z_{A,i} - 1$<br>$I^Z_{A,i} \rightarrow I^Z_{A,i} + 1$ | $\eta m_a I^Z_{A,i}$ |
| Neutral mutation in infected host set $I^Z_{A,I}$ | No change to compartments, frequency of neutral genotype sections updated | $\eta m_n I^Z_{A,i}$ |

**Antigenic similarity and cross-immunity**

In order to determine the immune cross-reaction between two strains it is necessary to determine the similarity between the antigenic sections of their bitstrings $G_1$ and $G_2$. We use the Hamming distance. This measure is defined as the number of bitstring locations in the two strains with different values. A Hamming distance of 0 indicates two strains are identical. A Hamming distance of $m_a$ indicates every element is different. The cross-immunity between strains $G_1$ and $G_2$ is the degree of similarity between antibodies to $G_1$ and antigens of $G_2$, or vice versa. It need not be the same as the genotypic similarity. Let the Hamming distance between $G_1$ and $G_2$ be $h$ and let the cross-immunity be given by $g(h)$, a monotonic increasing function. There is little empirical guidance to help determine this function but its form can have a significant impact on the evolutionary dynamics of the system. If $g$ is concave or linear, any number of antigenically distinguishable strains can coexist and selection for immune escape is weak. If $g$ is convex only a limited number of antigenic variants can coexist, and selection for immune escape is strong (4-5). In order to avoid creating immune selection simply from this functional form, we use a linear form $g(h) = \min\{\sigma h, 1\}$. The parameter $\sigma$ is the antigenic advantage associated with a single point mutation. We must cut off the function at 1 because cross-immunity greater than 1 would be cross-enhancement. This phenomenon has not been observed for influenza.

The function $g(h)$ maps genotypic similarity between two strains to immune cross-reaction. We now need to extend the framework to a function $f(A, G_1)$ that maps genotypic similarity between $G_1$ and a whole set of strains $A$ to cross immunity. In this case a host has been previously infected by all the strains in $A$ and has antibodies to all of those strains. The function $f$ determines how much protection those antibodies afford against a strain with antigenic genotype $G_1$. We combine these two concepts by defining $h_0$ to be the minimum Hamming distance between $G_1$ and each individual strain in $A$. We then define $f(A, G_1) = g(h_0)$ (2). This formulation assumes that only the strongest antibody reaction is relevant. An alternative method is to define $f(A, G_1)$ to be the product of all the individual Hamming distances (19). This formulation assumes that the antibodies function cooperatively and have a much more powerful effect in concert.

**Parameter values**

| Parameter | Meaning | Base value |
|---|---|---|
| $N$ | Population size of each region | $10^6$ |
| $\gamma$ | Recovery rate / year | 52 |
| $\mu$ | Natural death rate / year | 1/75 |
| $\delta$ | Amplitude of fluctuation in seasonal effective reproductive number | 0.4 |
| $\beta$ | Non-seasonal transmission intensity ($R_0 = \beta N/(\gamma + \mu)$) | $1.1N(\gamma + \mu)$ |
| $\sigma$ | Advantage associated with each antigenic genotype difference | 0 |
| $\tau_{YS}$ | Transmission coupling between seasonal and non-seasonal regions | 0.0005 |
| $\tau_{SS}$ | Transmission coupling between seasonal regions | 0.0005 |
| $\eta$ | Mutation rate / element / infected individual / year | 0.0004 |

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